B9

while 4 of the 9 genes repressed by MYC-ER increased in abundance more than two-fold. Therefore, genes identified by the conditional induction model discussed above also showed regulation in a physiological context. These findings support the conclusion that the identified genes, which are consistently regulated during both cell cycle progression and differentiation, are MYC target genes. - - -

Amendments to the specification are indicated in the attached "Marked Up Version of Amendments" (pages i - v).

REMARKS

Objection to the Specification

The Examiner has objected to the Specification because it contains an embedded hyperlink and/or other form of browser-executable code at page 18, line 16.

Applicants have amended the Specification to obviate this issue. The referenced hyperlinks, as amended, are no longer enabled. Therefore, reconsideration and withdrawal of the objection is respectfully requested.

Formal Drawings

The Examiner notes that the application was filed with informal drawings, and that Formal Drawings will be required when the application is allowed.

Applicants are submitting Formal Drawings concurrently herewith. Entry of the drawings is respectfully requested.

Rejection of Claims 13, 15 and 20 Under §102(b)

Claims 13, 15 and 20 are rejected under 35 U.S.C. §102(b) as being anticipated by Eilers *et al.* (1991. *EMBO J.*, 10:133-141; Reference U).

Applicants respectfully traverse this rejection. In order for a reference to anticipate under 35 U.S.C. 102, the reference must teach every aspect of the claimed invention either explicitly or impliedly. (MPEP 706.02). Applicants direct the Examiner's attention to steps (a) and (b) of Claim 13. They appear as follows:

"a) obtaining an indicator cell that expresses a chimeric receptor comprising MYC and a ligand binding domain"

and

"b) contacting the resulting indicator cell with an appropriate ligand in the presence and absence of an agent to be evaluated for its ability to regulate MYC's transcriptional regulation activity."

Although the Examiner asserts that Eilers *et al.* teach these two steps, Applicants point out that the claimed invention requires ***both*** an appropriate ligand ***and*** an agent to be evaluated. The ligand is not the same as the agent. Step (a) of Claim 13 clearly recites that the chimeric receptor comprises a "ligand binding domain"; thus, the "appropriate ligand" in step (b) is a ligand that binds the ligand binding domain of the chimeric receptor. The agent is not necessarily related to the receptor. The effect is that Claim 13 is directed to evaluating agents that affect the transcriptional regulation activity of MYC; it is not directed to the ability of a ligand to induce MYC expression or activity.

Eilers *et al.* teach the use of a ligand, *e.g.*, estrogen, to induce MYC activity, but they do not teach evaluation of a separate agent for effect on MYC transcriptional regulation activity. Eilers *et al.* teach the inducible expression of MYC and show that MYC has transcriptional regulation activity. In the inducible system taught by Eilers *et al.,* a fusion protein comprising MYC and an estrogen receptor ligand binding domain is used. The purpose of fusing MYC to the estrogen receptor is to allow the transcriptional regulation activity of MYC to be inducibly activated by the addition of estrogen or an estrogen analog. The estrogen receptor sequesters the fusion protein in the cytoplasm of a cell; MYC does not function as a transcriptional regulator in the cytoplasm. Upon contact with the appropriate ligand, *e.g.*, estrogen, the fusion protein is transported to the nucleus where MYC is active. Eilers *et al.* thus teach contacting a chimeric receptor comprising MYC with an appropriate ligand (*e.g.*, estrogen or an estrogen analog), but they do not teach contacting the chimeric receptor with an appropriate ligand ***in the presence and absence of an agent*** to be evaluated for its ability to regulate MYC's transcriptional regulation activity. By contacting the chimeric receptor with estrogen or an estrogen analog, Eilers *et al.* merely show the activation of MYC transcriptional regulation activity and are able to identify

genes that are regulated by MYC. Eilers *et al.* do not teach identifying agents that affect MYC's ability to regulate transcription.

Eilers *et al.* do not teach every step of the claimed invention; therefore, the teachings of Eilers *et al.* do not anticipate the invention of Claim 13. Claims 15 and 20 are dependent on Claim 13. Thus, the fact that Eilers *et al.* do not teach every step of Claim 13 similarly means that Eilers *et al.* do not teach every step of dependent Claims 15 and 20. Reconsideration and withdrawal of the rejection are respectfully requested.

<u>Rejection of Claims 13, 14, 17 and 20 Under §102(b)</u>

Claims 13, 14, 17 and 20 are rejected under 35 U.S.C. §102(b) as being anticipated by Lee *et al.* (1997. *Proc. Natl. Acad. Sci. USA*, 94:12886-12891).

Applicants respectfully traverse this rejection. In order for a reference to anticipate under 35 U.S.C. 102, the reference must teach every aspect of the claimed invention either explicitly or impliedly (MPEP 706.02). Although the Examiner asserts Lee *et al.* teach steps (a) and (b) of the invention of Claim 13, Applicants reiterate that the claimed invention requires ***both*** an appropriate ligand ***and*** an agent to be evaluated. The ligand is not the same as the agent.

The teachings of Lee *et al.* are similar to those of Eilers *et al.* discussed above. Lee *et al.* teach methods of inducing MYC activity by contacting a chimeric receptor comprising MYC and an estrogen receptor ligand binding domain with *e.g.*, an estrogen analog. Lee *et al.* teach the use of 4-OHT (an estrogen analog) to induce MYC activity, but they do not teach evaluation of a separate agent for effect on the transcriptional regulation activity of MYC. Therefore, the teachings of Lee *et al.* do not teach every step of the claimed invention, and therefore, the teachings of Lee *et al.* do not anticipate Applicants' claimed invention. Claims 14, 17 and 20 are dependent on Claim 13; thus, the fact that Lee *et al.* do not teach every step of Claim 13 similarly means that Lee *et al.* do not teach every step of dependent Claims 14, 17 and 20. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 16, 18 and 19 Under §103(a)

Claims 16, 18 and 19 are rejected under 35 U.S.C. §102(b) as being unpatentable over Eilers *et al.* in view of Zhu *et al.* (1998. *Proc. Natl. Acad. Sci. USA*, 95:14470-14475; Reference W).

Applicants respectfully traverse this rejection. "To establish a prima facie case of obviousness...the prior art reference (or references when combined) must teach or suggest all the claim limitations." (MPEP 706.02(j)). As discussed above, Eilers *et al.* do not teach every step of the claimed invention; specifically, Eilers *et al.* do not teach or suggest a method for evaluating an agent that regulates MYC-dependent transcriptional regulation of gene expression utilizing a ligand that binds to the ligand binding domain of the chimeric receptor and an agent to be assessed. Although Zhu *et al.* teach methods of evaluating gene expression using microarrays, they do not remedy this defect. Therefore, the combined references do not teach or suggest every step of the invention of Claim 13. Claims 16, 18 and 19 are dependent on Claim 13; thus, the teachings of Eilers *et al.* in light of Zhu *et al.* do not render dependent Claims 16, 18 and 19 obvious. Reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Replace the paragraph at page 9, lines 16 through 17, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

--- Figures 6A and 6B are [is a table]tables listing the 27 genes activated by MYC and the 9 genes repressed by MYC. Relative activation and repression levels are shown. ---

Please replace the paragraph at page 13, lines 3 through 12, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

--- Using oligonucleotide microarrays to monitor the effects of induced MYC expression, 27 target genes were found that are activated by MYC and 9 target genes were found that are repressed by MYC (see [Figure]Figures 6A and 6B). Based on changes in expression in the presence of cycloheximide, it was determined that most MYC target genes (18/27 of activated targets and 8/9 for repressed targets) are "direct targets," used herein to refer to target genes that are directly regulated by MYC and not by an intermediate transcription factor. This finding, coupled with the observation that none of the putative MYC target genes identified are transcription factors, argues against the idea that MYC's role is to activate a transcriptional cascade. Thus, the genes regulated by MYC are likely to be effector genes whose activities lead directly to specific cellular function. ---

Replace the paragraph at page 14, line 20 through page 15, line 7 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

--- A major effect of MYC on both *Drosophila* and mammalian cells is to increase the accumulation of cell size (Johnston, L. *et al.*, 1999. *Cell.* 98:779-790; Iritani, B. and Eisenman, R., 1999. *Proc. Natl. Acad. Sci. USA.* 96:13180-13185). Data described herein provide support for the

view that MYC directly influences cell size through protein synthesis. Earlier work had indicated that the rate-limiting translational initiation factor, EIF4E, is induced by MYC (Rosenwald, I. *et al.*, 1993. *Proc. Natl. Acad. Sci. USA.* 90:6175-6178). Work described herein indicates that MYC induces EIF5A, a translation initiation factor also thought to be involved in nucleocytoplasmic transport (Rosorius, O. *et al.*, 1999. *J. Cell Sci.* 112:2369-2380; Elfgang, C. *et al.*, 1999. *Proc. Natl. Acad. Sci. USA.* 96:6229-6234). Interestingly, MYC leads to increased levels of the previously identified target ornithine decarboxylase (Bello-Fernandez, C. *et al.*, 1993. *Proc. Natl. Acad. Sci. USA.* 90:7804-7808; Wagner, A. *et al.*, 1993. *Cell Growth Diff.* 4:879-883; [Figure]<u>Figures</u> 6<u>A and 6B</u>), which regulates a hypusine modification of EIF5A that is critical for its function (Park, M. *et al.*, 1998. *J. Biol. Chem.* 273:1677-1683). Other cell-size associated genes identified as MYC targets herein include several genes involved in nucleolar rRNA processing such as the structural proteins fibrillarin and nucleolin, the ribosomal protein RPS11, and EIF4γ. - - -

Replace the paragraph at page 18, line 15 through page 19, line 4 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

- - - A complete protocol for converting RNA into "target" suitable for hybridization to microarrays is available at <u>the world wide</u> web site [http://www.genome.wi.mit.edu/MPR]<u>genome.wi.mit.edu/MPR</u>. Briefly, polyA mRNA was selected with oligo-dT beads from total RNA extracted with Trizol reagent (Life Technologies, Gaithersburg, MD), and used to create cDNA with a T7-polyT primer and the reverse transcriptase Superscript II (Gibco-BRL, Gaithersburg, MD). Approximately 1 microgram of cDNA was subjected to *in vitro* transcription in the presence of biotinylated UTP and CTP. Target for hybridization was prepared by combining 40 micrograms of fragmented transcripts with sonicated herring sperm DNA (0.1 mg/mL) and 5 nM control oligonucleotide in a buffer containing 1.0 M NaCl, 10 mM Tris-HCl (pH 7.6) and 0.005% Triton X-100. Target was hybridized for 16 hours at 40°C to a set of four oligonucleotide arrays (HUM6000-1, HUM6000-2, HUM6000-3, HUM6000-4; Affymetrix, Santa Clara, CA) containing probes for 6416 human genes (5223 known human genes and 1193 unnamed ESTs). Arrays were washed at 50°C with 6X SSPET (0.9 M NaCl, 60 mM NaH_2O_4, 6 mM EDTA, .005% Triton X-100, pH 7.6), then at 40°C with 0.5X SSPET. Arrays were

then stained with streptavidin-phycoerythrein. Fluorescence intensities were captured with a laser confocal scanner (Affymetrix, Santa Clara, CA) and the Genechip software (Affymetrix, Santa Clara, CA). - - -

Replace the paragraph at page 21, lines 11 through 18, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

- - - [Figure]Figures 6Aand 6B summarize[s] the 27 genes that were up-regulated and 9 genes that were down-regulated in all three MYC induction experiments. This is a significantly greater number of genes than would be expected to be induced based exclusively on fluctuations due biological or technical variability. Several other previously reported MYC targets showed some evidence of regulation but did not meet our strict criterion of 2-fold induction in all three experiments. The complete data set for all of the experiments reported herein is available at the world wide web site [http://www.genome.wi.mit.edu/MPR]genome.wi.mit.edu/MPR, the teachings of which are incorporated herein by reference. - - -

Replace the paragraph at page 21, lines 19 through 23, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

- - - Significantly, only two of the genes identified in Figures 6A and 6B as putative MYC target genes have been previously reported as downstream MYC targets [ODC (Bello-Fernandez, C. *et al.*, 1993. *Proc. Natl. Acad. Sci. USA.* 90:7804-7808; Wagner, A. *et al.*, 1993. *Cell Growth Diff.* 4:879-883) and nucleolin (Greasley, P. *et al.*, 1999. *Nucl. Acids Res.* 28:446-453)]. - - -

Replace the paragraph at page 21, line 26 through page 22, line 6, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

- - - To discriminate between direct and indirect MYC targets, MYC-ER was activated in the presence of cycloheximide (Galaktionov, K. *et al.*, 1996. *Nature.* 382:511-517; Grandori, C. *et al.*, 1996. *EMBO J.* 15:4344-4357). By inhibiting protein synthesis, cycloheximide eliminated the possibility that MYC-induced proteins would subsequently modulate a secondary set of genes. Of the 27 genes consistently induced by MYC-ER, 18 genes (68%) were also up-regulated in the presence of cycloheximide, while almost all of the repressed genes (8/9) were also down-regulated under these conditions ([Figure]Figures 6A and 6B). These results suggest that most of the targets identified are likely to be direct targets of MYC. - - -

Replace the paragraph at page 22, lines 9 through 23, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

- - - To verify induction by an independent method, six induced target genes were chosen from the set of putative MYC target [gene] genes identified in [Figure]Figures 6A and 6B for Northern blot analysis. In all cases, the Northern blots confirmed the microarray results indicating up-regulation by MYC-ER. For four genes, the same RNA as was used for the microarray measurements was examined for two separate inductions, and for two genes RNA was investigated from an independent MYC-ER induction. As shown in Figures 3A-3C, FKBP52, FABP5, PPIF, EIF5A and cyclin D2 follow a similar pattern of expression to that of the known target gene ODC. The ratio of transcript levels in MYC-ER expressing fibroblasts with and without stimulation determined by Northern blot correlated well with the estimates based on the microarrays: 2.3 (Northern, exp. 1)/2.3 (microarray, exp. 1) and 2.2 (Northern, exp. 2)/2.1 (microarray exp. 2) for FKBP52; 1.8/2.0 and 1.4/2.1 for PPIF; 4.1/3.6 for FABP5; 1.8/2.3-3.0 for EIF5A and 3.5/2.2-5.7 for cyclin D2 (Figures 3A and B). Thus, the Northern blot data demonstrate an increase in expression in the same range as expected from the microarray results for all of the genes tested. - - -

Replace the paragraph at page 23, lines 6 through 19, with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

- - - In order to determine whether the putative targets identified in the microarray assays are influenced by changes in MYC levels under physiologically relevant conditions, it was assessed whether these targets are also affected during the shut-off of endogenous MYC which accompanies hematopoietic differentiation (Henriksson, M. and Luscher, B., 1996. *Adv. Cancer Res.* 68:109-182 1996). In [Figure]Figures 6A and 6B, ratios of gene expression in differentiated and undifferentiated HL60 cells are given for each of the genes identified as a candidate MYC target in the MYC-ER experiments. Seventeen of the 27 genes consistently induced in the MYC-ER experiments showed a greater than 2-fold decline in expression as HL-60 cells differentiated, while 4 of the 9 genes repressed by MYC-ER increased in abundance more than two-fold. Therefore, genes identified by the conditional induction model discussed above also showed regulation in a physiological context. These findings support the conclusion that the identified genes, which are consistently regulated during both cell cycle progression and differentiation, are MYC target genes. - - -